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TITLE: Accelerate Genomic Aging in Congenital Neutropenia.

PRINCIPAL INVESTIGATOR: Dr. Daniel C Link

CONTRACTING ORGANIZATION: Washington University  
Saint Louis, MO 63130-4862

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14. ABSTRACT: The goal of this research is to define the molecular mechanisms responsible for the markedly increased risk of transformation to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) in patients with congenital neutropenia. We hypothesize that replicative stress and/or changes in the bone marrow microenvironment in patients with congenital neutropenia leads to a higher rate of accumulation of mutations in hematopoietic stem/progenitor cells (HSPCs), which, in turn, contributes to transformation to MDS/AML. We further hypothesize that G-CSF treatment accelerates the accumulation of mutations in HSPCs. Finally, we hypothesize that truncation mutations of <i>CSF3R</i> , which are common in patients with severe congenital neutropenia (SCN) and are associated with increased G-CSF signaling and transformation to MDS/AML, accentuate the rate of mutation accumulation. We will test these hypotheses in the following Specific Aims. <u>Aim 1. To determine whether HSPCs undergo premature genomic aging in SCN or SDS.</u> We will measure the mutation burden in individual HSPCs from patients with SCN, Shwachman-Diamond syndrome (SDS), cyclic neutropenia, or age-matched healthy controls. <u>Aim 2. To determine whether increased G-CSF signaling accelerates the mutation rate in HSPCs.</u> Here, we will assess the impact of prolonged (6 month) G-CSF therapy on HPSC mutation burden in mice. These data will provide novel insight into the mechanisms of leukemic transformation in CN. They also should provide new insight into the safety of long-term G-CSF therapy in CN. Finally, our novel assay to measure mutation burden in HSPCs may provide an approach to assess DNA damage after exposure to genotoxic agents, such as radiation.					
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## 1. INTRODUCTION

The goal of this research is to define the molecular mechanisms responsible for the markedly increased risk of transformation to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) in patients with congenital neutropenia. We hypothesize that replicative stress and/or changes in the bone marrow microenvironment in patients with congenital neutropenia leads to a higher rate of accumulation of mutations in hematopoietic stem/progenitor cells (HSPCs), which, in turn, contributes to transformation to MDS/AML. We further hypothesize that G-CSF treatment accelerates the accumulation of mutations in HSPCs. Finally, we hypothesize that truncation mutations of *CSF3R*, which are common in patients with severe congenital neutropenia (SCN) and are associated with increased G-CSF signaling and transformation to MDS/AML, accentuate the rate of mutation accumulation. We will test these hypotheses in the following Specific Aims. Aim 1. To determine whether HSPCs undergo premature genomic aging in SCN or SDS. We will measure the mutation burden in individual HSPCs from patients with SCN, Shwachman-Diamond syndrome (SDS), cyclic neutropenia, or age-matched healthy controls. Aim 2. To determine whether increased G-CSF signaling accelerates the mutation rate in HSPCs. Here, we will assess the impact of prolonged (6 month) G-CSF therapy on HPSC mutation burden in mice. These data will provide novel insight into the mechanisms of leukemic transformation in CN. They also should provide new insight into the safety of long-term G-CSF therapy in CN. Finally, our novel assay to measure mutation burden in HSPCs may provide an approach to assess DNA damage after exposure to genotoxic agents, such as radiation.

## 2. KEYWORDS

Congenital neutropenia  
Severe congenital neutropenia  
Shwachman Diamond syndrome  
Cyclic neutropenia  
Hematopoietic stem cells  
Granulocyte colony-stimulating factor  
Granulocyte colony-stimulating factor receptor  
Acute myeloid leukemia  
Myelodysplastic syndrome

## 3. ACCOMPLISHMENTS

The major goals and objectives of this research remain the same as originally proposed. Progress and plans for each of the tasks proposed Statement of Work (as updated in our no cost extension approved 4-5-2016) are detailed below.

**Task 1. To determine whether HSPCs undergo premature genomic aging in congenital neutropenia (Timeframe: 1-36 months).** In this task, we will assess the mutation burden in individual hematopoietic stem/progenitor cell (HSPC) clones obtained from healthy controls or patients with congenital neutropenia.

1a. Obtain human studies approval for whole exome sequencing of healthy controls (Timeframe 1-2 months; completed). Human studies approval at Washington University, University of Michigan, and the University of Washington have been obtained.

1b. Obtain human studies approval for studies from the DoD Human Research Projection Office (Time frame 1-3 months; completed). DoD approval has been obtained.

1c. Obtain human blood or bone marrow samples from patients with SDS, SCN, cyclic neutropenia, or healthy controls (Timeframe 3-24 months, completed). We proposed to obtain a total of 15 bone marrow of blood samples each from patients SDS, SCN, cyclic neutropenia, or age-matched healthy controls. We reached or exceeded this goal for each cohort except cyclic neutropenia (see Table 1).

1d. Generate hematopoietic stem/progenitor cell (HSPC) colonies from patients with CN or healthy controls (Timeframe 3-24 months; completed). We encountered some technical difficulty in expanding HSPC colonies. In most cases, this was related to poor sample quality. The final summary of successful HSPC colony expansions is shown in Table 1.

**Table 1. Human HSPC expansion**

<b>Sample type</b>	<b>Total # of samples</b>	<b>Successful HSPC expansion</b>	<b>Successful HSPC Sequencing</b>	<b>Ongoing CHIP Sequencing</b>
Normal	21	9	9	21
SCN	25	13	13	25
SDS	15	4	4	15
Cyclic	9	5	5	8

The total number of blood or bone marrow samples obtained is shown.

The number of successful HSPC expansions and HSPC clone sequencing is shown

The number of blood/bone marrow samples undergoing CHIP sequencing is shown

1e. Sequence HSPC clones and bone marrow fibroblasts (Timeframe 12-30 months, ongoing). The number of samples that have successfully undergone HSPC sequencing are shown in Table 1. Samples that are being sequenced to determine the incidence of clonal hematopoiesis (CHIP) also are shown in Table 1. This task should be completed in the next 3 months.

1f. Analysis of the sequence data (Timeframe 15-33 months, ongoing). It should take approximately 4 weeks to analyze the data.

1g. Validation of mutations (Timeframe 18-36 months, planned). All somatic mutations identified by sequencing will be validated by generating custom capture reagents and sequencing the HSPC clone and bone marrow fibroblast DNA again. This takes approximately 3 months.

**Task 2. To determine whether increased G-CSF signaling accelerates the mutation rate in HSPCs (Timeframe 1-21 months, completed).** In this task, we will assess the mutation burden in individual murine HSPC clones exposed to G-CSF treatment for 6 months. These analyses were performed in wildtype and *Csf3r* mutant mice. Results are summarized in Figure 1.

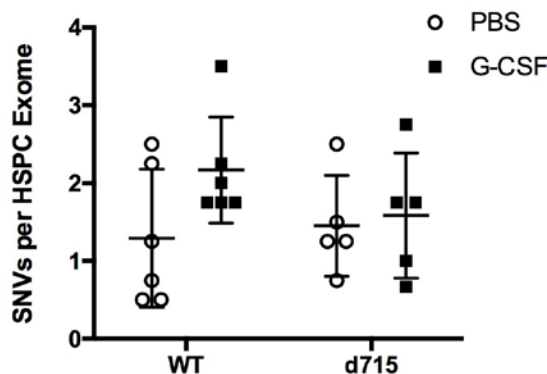
2a. Obtain regulatory approval from the DOD Animal Care and Use Review Office (**Timeframe 2 months, completed**). DoD Animal Care approval has been obtained. This task is complete.

2c. Generate HSPC colonies from mice (**Timeframe 5-14 months, completed**). We have successfully generated HSPC colonies from 27 wildtype or *Csf3r* mutant mice treated with G-CSF or saline alone for 6 months.

2d. Sequence HSPC clones (**Timeframe: 12-16 months, completed**). Sequencing of HSPC clones from a total of 22 mice is completed. Data are summarized in Figure 1.

2e. Analysis of the sequence data (**Timeframe 14-18 months, completed**). This task is complete. Data are summarized in Figure 1.

2f. Validation of mutations (**Timeframe 14-21 months, completed**). This task is complete. Data are summarized in Figure 1.



*Figure 1. Mutation burden in murine HSPCs.* Wild-type (WT) or *Csf3r*<sup>d715/d715</sup> (d715) mice were treated with pegylated G-CSF (1 mg/kg three times per week) for 6 months. Single Kit<sup>+</sup> lineage- hematopoietic stem/progenitor cells were sorted and expanded over a 3 week period with stromal cell support. The exomes of a minimum of 3 HSPC colonies along with matching tail DNA were sequenced. Shown is the average number of somatic single nucleotide mutations or indels per exome per mouse (each data point represents the average of at least 3 HSPC colonies). Neither treatment with G-CSF nor the presence of

truncating (activating) mutations of the G-CSF receptor (*Csf3r*) were associated with a difference in HSPC mutation burden.

**Opportunities for training and professional development:** nothing to report

**Dissemination of results to communities of interest:** These results will be submitted for presentation at the annual meeting of the American Society of Hematology. We anticipate submitting a manuscript detailing these findings in the next 6-9 months.

**Plans for the next reporting cycle.** All tasks will be completed in this reporting cycle.

#### 4. IMPACT

Impact on the development of the principal discipline: We have optimized the approach to culture and expand both human and murine HSPCs clones. This technique will be of use to investigators in the field studying clonal architecture and clonal evolution in hematopoiesis.

Our data suggest that prolonged G-CSF treatment is not associated with an increase in HSPC mutation burden.

Impact on other disciplines: nothing to report

Impact on technology transfer: nothing to report

Impact on society: nothing to report

## 5. CHANGES/PROBLEMS

Changes in approach: nothing to report

Actual or anticipated problems: nothing to report

Significant changes in the use or care of human subjects, vertebrate animals, biohazards, and/or select agents: nothing to report

## 6. PRODUCTS

Nothing to report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals worked on this project:

Name:	Daniel C. Link
Project Role:	Principal Investigator
Researcher Identifier:	xxxxxx
Nearest person month involved:	1 month
Contribution to Project:	no change
Funding Support:	no change

Name:	Jun Xia
Project Role:	Staff Scientist
Researcher Identifier:	xxxxxx
Nearest person month involved:	3 months
Contribution to Project:	no change
Funding Support:	no change

Changes in active or other support of the PD/PI:

RO1 HL131655-01 (Link)	4/1/16-3/31/2021	2.00 CAL
National Institutes of Health		
Regulation of hematopoietic niches by TGF family signaling		

The major goals of this project are to define the role of TGF family signaling in the development and maintenance of hematopoietic niches. There is no overlap with the DoD proposal.

1R01CA194552-01 (DiPersio, PI) 04/01/15-03/31/20

NIH/NCI

Title: Retargeting Agents to Treat AML

This is a recently awarded grant on which I serve as a collaborator (0.20 CAL). There is no overlap with the DoD Proposal.

TRA 6030-10 (Link, PI) 10/1/09-9/30/2013 2.00 CAL

Leukemia & Lymphoma Society

Mutational Profiling of microRNAs in t-AML/t-MDS This grant is completed

RO1 CA136617 (Link, PI) 5/1/09-4/31/14 1.80 CAL

National Institutes of Health

Clonal Dominance of Hematopoietic Stem Cells Expressing Mutant CSF3R

This grant is completed

7539-55 (Link, PI) 1/1/2012-12/30/2013 0.60 CAL

Barnes-Jewish Hospital Foundation, Cancer Frontier Grant

Targeting the bone marrow microenvironment in multiple myeloma

This grant is completed

What other organizations are involved as partners:

Organization Name: The University of Washington

Location of Organization: Seattle, Washington

Partner's contribution to the project: Drs. Dale and Shimamura provide coded human blood or bone marrow samples from patients with congenital neutropenia.

No changes

Organization Name: University of Michigan

Location of Organization: Ann Arbor, MI

Partner's contribution to the project: Dr. Larry Boxer provide coded human blood or bone marrow samples from patients with congenital neutropenia.

No changes

**8. SPECIAL REPORTING REQUIREMENTS:** None

**9. APPENDICES:** None